Neuroscience Letters 523 (2012) 131–134

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Digitized quantitative electroencephalographic patterns applied as magnetic fields inhibit melanoma cell proliferation in culture

Lukasz M. Karbowski[a,b], Sean L. Harribance[c], Carly A. Buckner[d], Bryce P. Mulligan[b], Stanley A. Koren[b], Robert M. Lafrenie[a,b,d,e], Michael A. Persinger[a,b,d,*]

[a] Department of Biology, Laurentian University, Sudbury, Ontario, Canada
[b] Behavioural Neuroscience Program, Laurentian University, Sudbury, Ontario, Canada
[c] Sugarland, Texas, United States
[d] Biomolecular Sciences Program, Laurentian University, Sudbury, Ontario, Canada
[e] Regional Cancer Program, Sudbury Regional Hospital, Sudbury, Ontario, Canada

HIGHLIGHTS

- Digitized EEG patterns applied as magnetic fields slow cell growth.
- Specific brain patterns slowed melanoma cell growth in culture.
- Spectral power of effective pattern had predictable effects.
- Fine temporal structure of EM fields critical for biological effects.
- Effect evoked with weak intensities and one hour daily exposures.

ARTICLE INFO

Article history:
Received 31 March 2012
Received in revised form 18 June 2012
Accepted 22 June 2012

Keywords:
Melanoma
Magnetic fields
Physiological patterns
Electroencephalographic activity
Right temporal lobe

ABSTRACT

Weak (1 μT) physiologically patterned magnetic fields produce changes in behavioral, physiological, and cellular activity. In the present experiments 12 temporal samples of the electroencephalographic anomaly and normal activity of a person (SLH) whose proximity reliably affected the brain activity of others were extracted from QEEG data, digitized, and presented as equivalent magnetic field patterns to B16 mouse melanoma cells. Only two of the patterns, both originating from the primary source (right temporal lobe) of the EEG anomaly reduced the cell growth by one-third compared to the other patterns extracted from his QEEG or sham field exposures. In previous experiments these EEG transients were also associated with marked increases in photon emissions from the right side of SLH's head. The results suggest that the intrinsic complexity of electroencephalographic patterns of some people, when amplified appropriately and applied as computer-generated magnetic fields in the three spatial planes, could diminish cancer cell growth.

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Whole body or cerebrally focused exposures to physiologically patterned magnetic fields [20] within the range of background power levels (~1 μT) have been shown to reliably affect analgesia and more complex behaviors in invertebrates [16,19], rodents [2,10] and human subjects [12]. That these fields affect classic cell receptors is suggested by synergistic interactions with agonists and antagonists of specific receptor subtypes [9]. During periods of physiological extremes, such as the enhanced metabolic activity correlated with electrical seizures, non-linear intensity effects upon vitality have been measured [9]. Conspicuous increases in mortality occurred if rat pups had been exposed perinatally to 7 Hz amplitude modulated fields with strengths ~5 nT but not too lesser or great intensities [17].

Most "physiological" patterns have been derived from theoretical or crude imitations of natural conditions. However with modern quantitative electroencephalographic (QEEG) technology, EEG patterns can be digitized and formatted to be presented by computer-based systems as complex temporal patterns of magnetic fields to target systems [7]. In light of the implication that extremely low frequency magnetic fields [3], particularly 50 Hz and 60 Hz power frequencies, can affect the dynamics of cancer [18], we have been investigating the optimal parameters by which frequency and amplitude-modulated, biofrequency magnetic fields can *slow* or *cease* the rate of melanoma (skin cancer) cells. Recent

* Corresponding author at: Behavioural Neuroscience Program, Departments of Psychology and Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6. Tel.: +1 705 675 4824; fax: +1 705 671 3844.
E-mail address: mpersinger@laurentian.ca (M.A. Persinger).

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http://dx.doi.org/10.1016/j.neulet.2012.06.059

experiments by Buckner [1] have shown that specific patterns of these fields inhibited growth in three different lines of cancer cells (human and non-human) but did not affect three different lines of normal cells. A series of biomolecular experiments over two years indicated the patterned magnetic fields increased ERK phosphorylation, thus increasing inhibition of cell proliferation, and affected T-type calcium channels. Increases in calcium concentrations activate several G-proteins, such as Protein Kinase C (PKC) and Calmodulin MAPK, which in turn affect the signaling pathways such as ERK. The specific magnetic field pattern that produced the inhibition of melanoma cell proliferation was the same field pattern that diminished the growth of melanoma cells after their injection into C57 mice [4].

SLH's unusual capacity to infer accurately the memories and experiences of people proximal to him is strongly correlated with the emergence of a cerebral anomaly within his right hemisphere that has been measured by multiple quantitative electroencephalographic (QEEG) and single photon emission computerized tomography (SPECT) methods [5,13]. The "Harribance Configuration" (HC), that has been analyzed in great detail elsewhere [5], is a brief (a few seconds) gamma (30–40 Hz) pattern over the right temporofrontal regions. Spectral analyses showed a major power enhancement of around 20 Hz over the right frontocentral and temporal lobe; these higher frequencies were superimposed upon an elevation in theta-band (4–8 Hz) power over the right temporal lobe only. Low resolution electromagnetic tomography (s_LORETA) revealed the increased activation was also localized within the right temporal lobe but extended into the adjacent insula. Here we present clear evidence that SLH's specific anomalous EEG pattern, *when applied as an electromagnetic field* to cell cultures during standard conditions, significantly impaired the growth of mouse melanoma cells.

Twelve 30-s samples of SHL's [5] electroencephalographic activity were obtained from the specific recordings from locations F7, F8, T3, and T4. These areas were selected because T4 and F8 are associated with the cerebral regions associated with the HC [13] while F7 and T3 served as intracerebral controls. The EEG data had been recorded during SLH's visit to the laboratory two years ago [5] on a Mistar-201 portable QEEG equipped with a 19-channel sensor cap (Electrode Cap International) which was patterned according to the International (10–20) Standard of Electrode Placement. Impedance for all channels was maintained under 10 kΩ and data were acquired continuously by WinEEG software with a sampling rate of 250 Hz.

All classical sensor placements over the left hemisphere were normal for the person's age and the correlative activity, except for the anomalous area generating the HC. The duration of this pattern in the presence of another person was shown to be strongly correlated with his accuracy of intuition concerning the medical history of this person as verified by blind ratings [5].

The digitized data for each of the 12 samples obtained two years ago were then transformed by software so that the min–max ranges in μV from the raw stored quantitative data were standardized to integer values between 0 and 256. These values were transformed by a customized digital-to-analog (DAC) converter (U.S. Patent 6,312,376 B1: November 6, 2001; Canadian Patent No: 2214296) from −5 to +5 V (127 = 0 V). The pattern was translated so that maximum fidelity occurred when each number between 0 and 256 was programmed for a point duration (the time the number, *i.e.*, discrete voltage, activates the solenoids) of 3 ms. Consequently, because each voltage point from the raw QEEG data was 4 ms (250 Hz sampling), there was potential distortion of the absolute pattern. The 3 ms point duration was selected because multiple experiments by Buckner [1] employing the same frequency-modulated magnetic field that produced analgesia in rats [10] reduced melanoma cell growth by 50% for both *in vivo* and *in vitro* exposures. Point durations of 1, 2, 4, or 5 ms were not effective [1].

Each of the 12 samples contained a sequence of 1132 numbers (again each between 0 and 256) that created the specific pattern. These DOS-based DAC files were transferred to a 286 laptop computer. The port times for this model and specific timing circuitry are optimally compatible with the algorithms for the software. The numbers were converted through our software (©Complex 2.2, Stan A. Koren, October 2003) by one of the custom-constructed digital-to-analog converters and then delivered to the same custom constructed exposure box employed in other studies for more traditionally generated magnetic field patterns [1]. To this 10 cm × 10 cm × 10 cm plastic box, six (3 pairs) of solenoids (modified reed switches) were affixed midway along each plane and connected so that the applied patterned magnetic field was generated simultaneously in each of the three spatial planes [11]. Each solenoid was 50 Ω, embedded with a small iron nail, and contained within a plastic (film) canister. The efficacy of this configuration has been shown many times [11]. The plates (Sarstedt 60 × 14 mm) containing the cells occupied the middle of the inside of the box.

B16-BL6 murine melanoma cells were cultured *in vitro* in DMEM containing 10% fetal bovine serum and antibiotics in a CO_2 incubator at 37°. Plated cells were exposed to only one of the 12 field patterns or to sham field conditions for 1 h per day for 5 successive days using the same procedure that revealed the cell-growth reducing effect in melanoma cells from similar intensity physiologically patterned magnetic fields [1]. The cells exposed to all patterns or sham field conditions were at the same state of confluence.

Cell cultures in the sham condition were placed in the incubator without the fields being present (except the background 60 Hz from the incubator that was present in all conditions). Each day, after exposure, one of the plates was harvested to observe the effects of the specific pattern upon cell proliferation. Cells were harvested following incubation in trypsin and collected by centrifugation at 400 × *g* for 10 min. The cell pellets were re-suspended in PBS containing 0.1% trypan blue. Quantification was completed by using a hemacytometer. For each sampled plate, eight separate counts of live cells were obtained and averaged. All field patterns were tested at least twice and triplicates were completed for the two most effective patterns. The cytometrist was blind to the origin of the magnetic field patterns.

The mean cell counts for the 2–3 replicates for each of the 12 patterns were averaged. Means and standard deviations were calculated and graphed. To discern the distinctive characteristics of two effective patterns spectral analyses were completed for each sequence. The spectra for the 10 non-effective patterns were averaged. The spectra for each of the two effective patterns as well as the average were subtracted from the value for the 10 non-effective patterns. The absolute value was then plotted as a function of frequency (to the nearest 0.01 Hz), as revealed by the output, to discern the threshold for divergence.

The results were conspicuously clear (Fig. 1). Only the two patterns (replicated in triplicate for each) extracted from SLH's *right* temporal region, the one most correlated with his remarkably accurate intuition about others and whose presence was associated with a gradual congruence of the EEG of the person proximal to him [14], produced a significant drop in the growth rate of the melanoma cells. As shown in Fig. 1, the other 10 patterns were not reliably effective or not effective at all and did not differ from sham-field exposures. When plotted over time the cell values for the other 10 patterns were so closed to each other and the sham field conditions that the identities of the different lines were not visually discriminable. The proportion of the decrease growth relative to sham field conditions for the two effective magnetic field patterns from a replication of the effect is shown in Fig. 2.

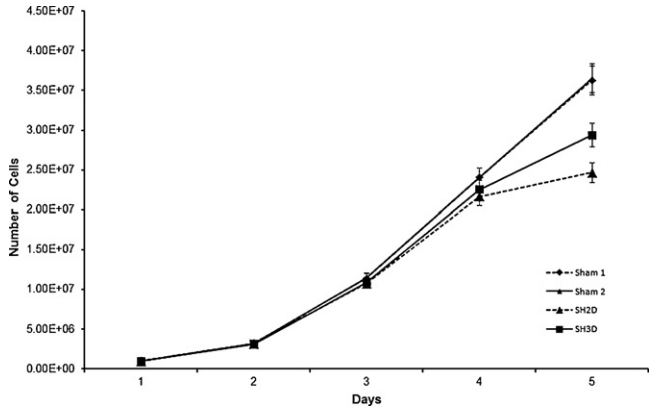

Fig. 1. Mean numbers of cells per plate as a function of days of 1 h exposures to the magnetic field sequences extracted as patterns (SH2D, SH3D) from SLH's right temporal lobe EEG activity compared to sham-field (Sham 1,2) exposures. Effects from magnetic field sequences extracted from EEG patterns from other regions did not differ from sham-field controls. Vertical bars indicate standard errors of the mean (SEM).

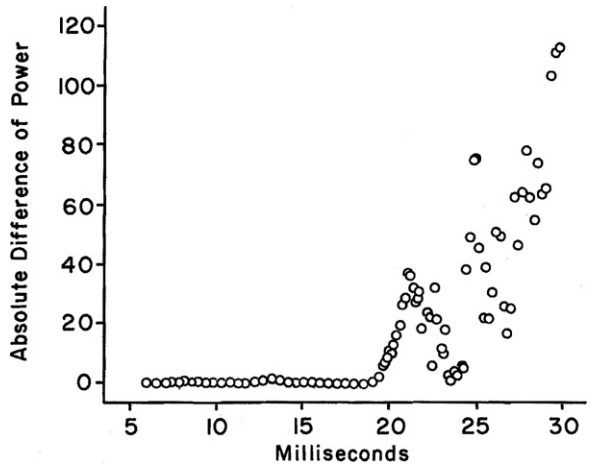

Fig. 3. Results of relative spectral power differences between the two averaged effective magnetic field patterns digitized from SLH's right temporal EEG activity and the non-effective patterns from the other regions of his brain; the duration in ms (inverse Hz).

Spectral analyses for the two effective patterns indicated that each differed significantly from the mean of the 10 other non-effective patterns for power levels for intervals between 20 ms and 30 ms (33–50 Hz) which is within the gamma range for EEG activity. As indicated in Fig. 3, the absolute values for the difference for superimposed power spectra for the averages of the two effective patterns deviated at frequencies below ~50 Hz. In other words, compared to the 10 non-effective patterns, there was more relative power within the 33–50 Hz range embedded within the EEG segments that suppressed the melanoma growth. The correlation (Spearman rho and Pearson *r*) for the units of the power spectra between the two effective patterns for the successive 0.1 ms increments for the narrow range between 19.5 and 21.5 ms was >0.95. Correlation coefficients for all other ranges as well as the total range were clustered around absolute values of 0–10.

That physiologically patterned magnetic fields, simulated or directly generated by living systems, affect cells has strong empirical support [1]. Like the syntax of a sentence relative to its loudness, the pattern of the field appears to be more important than the intensity for communicating information. In our experiments direct application as a magnetic pattern of an extraction of SLH's anomalous EEG pattern (HC), whose occurrences have been quantitatively associated with conspicuous modification of other people's QEEG patterns when they sat near him [5,13], inhibited the proliferation rate of cancer cells if they were exposed to this pattern for one hour per day for five successive days. Magnetic field patterns generated from QEEG data from areas of SLH's brain that were not correlated with this proximal effect [5,13] upon others did not influence melanoma cell growth. The one hour per day exposures to the right temporal lobe-derived magnetic field patterns conspicuously suppressed growth after 3 days (3 h) of exposure. However, the effect was not as strong as that induced by more repetitive, frequency-modulated field which suppressed melanoma growth by 50% [1]. It is possible we did not extract and test the most potent configuration from the QEEG samples.

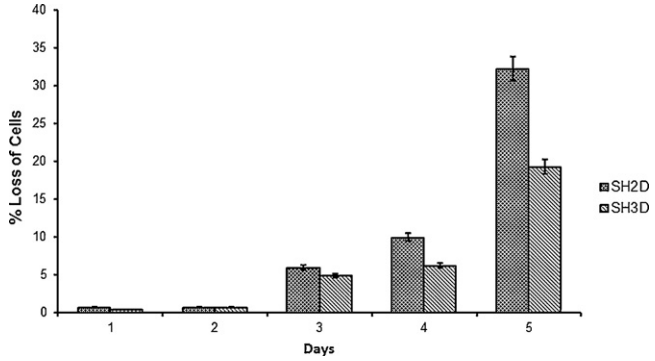

Fig. 2. Percent loss of cells exposed to the two effective patterns compared to sham field values (that did not differ significantly from the other 10 digital patterns extracted from the normal regions of the cerebrum) over days for a replication experiment. Vertical bars indicate SEMs.

Spectral analysis indicated that the two effective patterns contained more relative power within the "40 Hz" band associated with the generation of consciousness [6,8]. The action at a distance issue may be less contentious considering the reliable measurement of copious ultraweak photon emissions from SLH's right hemisphere (the area containing the anomaly) that was concurrent with minute but recordable decreases in components of the adjacent earth's static magnetic field. These changes, with magnitudes around 5–10 nT, occurred up to one meter away from SHL when the configuration was being displayed [5,13]. The potential utility of neuroquantum approaches [14,15] is apparent.

We suggest the molecular mechanisms associated with the inhibition of cell growth from application of the specific HC patterns could be similar to those identified by Buckner [1] for similar frequency-modulated patterns. Because cells typically display changes in membrane potentials as a function of various stages of the cell cycle, these fields might alter calcium influx and arrest or delay the cell cycle. However Buckner [1] found that following cell cycle synchronization using thymidine blockers, the fields she employed did not arrest the cycle. Direct effect upon processes that initiate changes in cell surface receptors, T-type calcium channels, or G proteins may lead to alterations in signaling pathways downstream.

Acknowledgments

Thanks to Professor Kevin Saroka for comments. This research was supported in part by Dr. E. Bosarge, Jr. Chairman, Quantlab LLC.

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